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ULTRASTRUCTURAL STUDIES ON NICOTIANA TABACUM POLLEN TUBES GROWN IN DIFFERENT CULTURE MEDIUM (PRELIMINARY RESULTS).*

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SUMMARY

The effects of four different culture media on the growth of the pollen tube of *Nicotiana tabacum* were investigated. Ultratructural examination revealed that the different media used did not provoke significant morphological differences. Substantial differences were observed in the percentage of generative cell division. Data on the presence of microtubules in the generative and vegetative cells are also reported.

1. INTRODUCTION

In the last decades many incubation media for the germination of pollen have been adopted (see STANLEY & LINSKENS 1974). Recently, further culture media have been studied, which, while giving the same germination percentage, promote the tube growth considerably more than the conventional media (HONG-QI & CROES 1982) also permitting a high frequency of generative cell division and sperm cell formation (LAFLEUR et al. 1981).

The purpose of this study was to identify the optimal culture medium for *Nicotiana* which would permit both good morphological preservation of the tube cytoplasm and a high frequency of generative cell division, with the future aim to isolate the sperm cells for biochemical and immunological studies.

2. MATERIALS AND METHODS

Plants of *Nicotiana tabacum* were grown in a greenhouse with natural temperature and light. Freshly collected pollen was sown in 35 × 10 mm Petri dishes at room temperature without shaking. The density of the pollen was 3 mg (fresh weight) per ml. The following culture media were used:
PEG-medium (Poly-ethylene glycol-400) according to HONG-QI & CROES (1982)
BK-medium (BREWBAKER & KWACK 1963, 1964)
M-medium (MASCARENHAS 1966)
W-medium (WALKER 1957).

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Pollen tubes were collected 2, 3, 4, 5 and 6 hours after sowing. The ultrastructural observations, here reported, were made 4 and 5 hours after sowing. The samples were fixed in 3% GA in 0.075 Cacodylate buffer, pH 7.2 for 30 min. They were then washed in buffer and postfixed in a buffered solution of 2% osmium tetroxide for 1 h. After washing in bidistilled water and poststaining in an aqueous solution of 2% Uranyl acetate, pollens were dehydrated in an ethanol series and embedded in Spurr's low viscosity resin (SPURR 1969). Sections were cut with a diamond knife and observed by JEOL Jem 100B electron microscope at 80kV.

3. RESULTS

The used culture media were liquid, so the hydration took place as soon as the pollen was sown. Pollen tubes generally appeared 45–55 min. after seedling. Germination was high in PEG (90–92%) and BK (88–90%) and slightly lower in W (80–82%) and M (85–87%). Pollen tube length, 6 hours after sowing, was practically the same in PEG and BK and less in W and M. Generative cell division was 18–20% in M, slightly less in W (10–12%) and completely unsatisfactory in PEG (4–6%) and BK (6–8%).

Although the pollen tubes grow at different rates according to the used culture medium, the functional (apical, sub apical nuclear and vacuolization and callose plug formation zones) zones previously described in *Nicotiana* and other species (CRESTI et al. 1977, CRESTI et al. 1980, CRESTI et al. 1985) are still clearly evident.

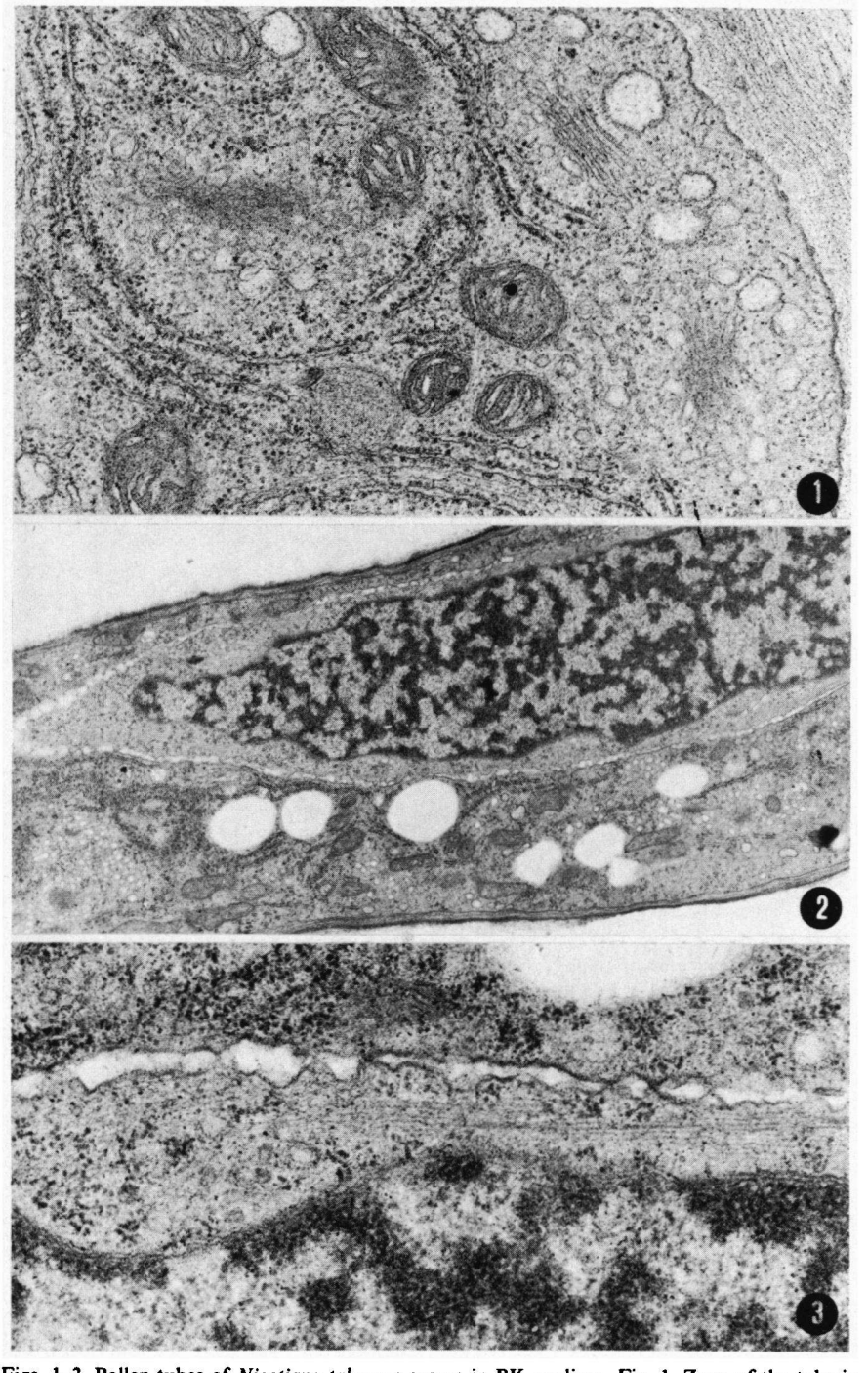
Ultrastructural description of pollen tubes grown in the different media, with particular reference to the preservation of the generative cell, walls and plasma membrane are reported.

3.1. BK-medium

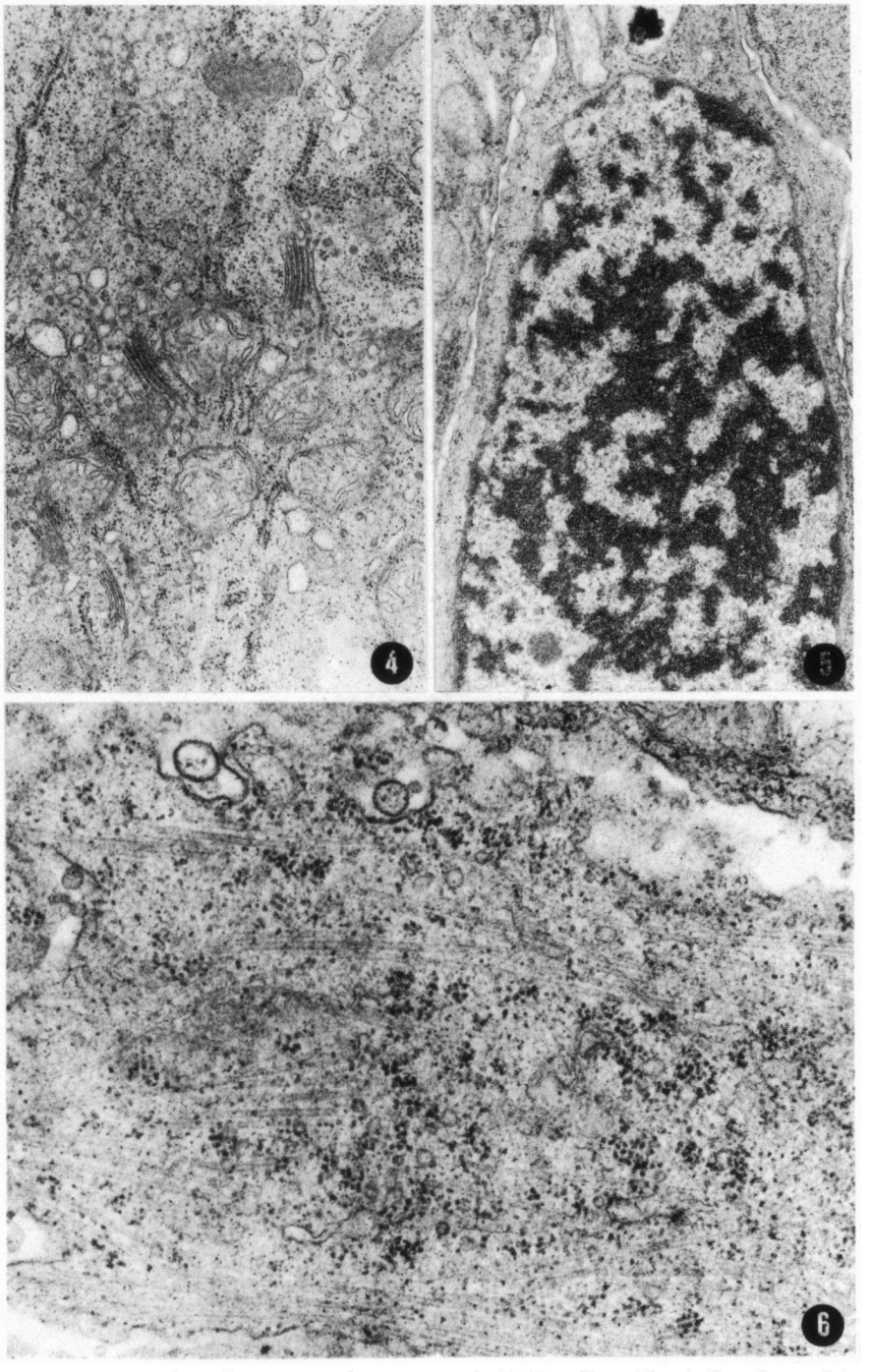
The pectocellulosic and callosic walls of the pollen tubes were clearly evident except in the apical zone where the callosic wall was absent (*fig. 1*). The generative cell wall profile was not uniform but had a vesicular appearance in places (*fig. 2*). The generative cytoplasm was less electrondense than the vegetative cytoplasm. In the generative cell, the microtubules were clearly visible from the moment of pollen tube emission; their aggregation in bundles became marked, when the cell had completely penetrated in to the tube (*fig. 3*). Microtubules were not observed in the vegetative cytoplasm.

3.2. PEG-medium

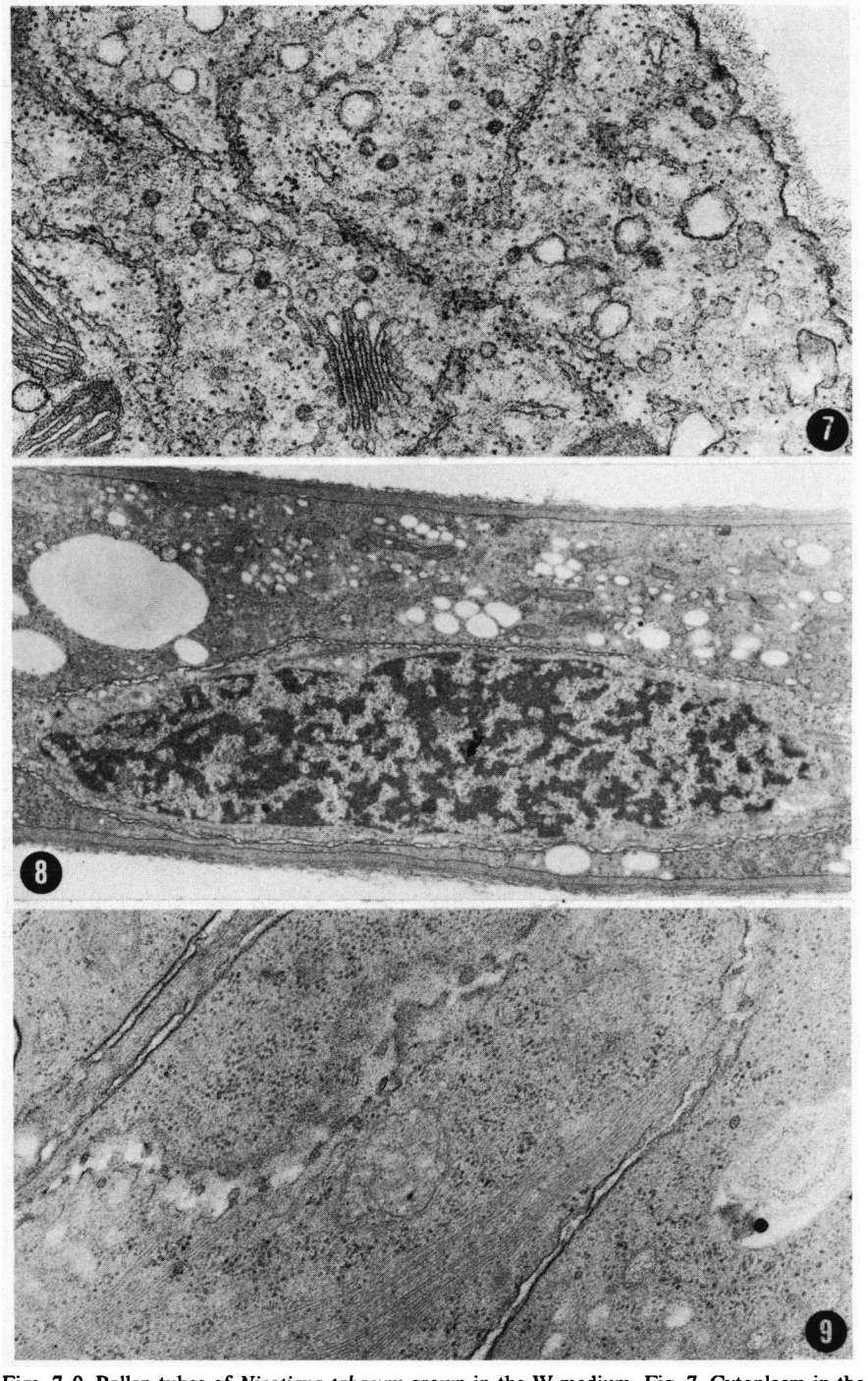
The tubes were of uniform growth but the apex was slightly enlarged. The pectocellulosic and callosic walls were clearly visible. In the apical zone the pectocellulosic wall was very thin and the plasma membrane uniform. The cell organelles appeared to be well preserved (*fig. 4*). The generative cell wall was uniform but occasional contact between the two membranes was observed (*fig. 5*). The generative cytoplasm was of similar electron density to the vegetative cytoplasm.



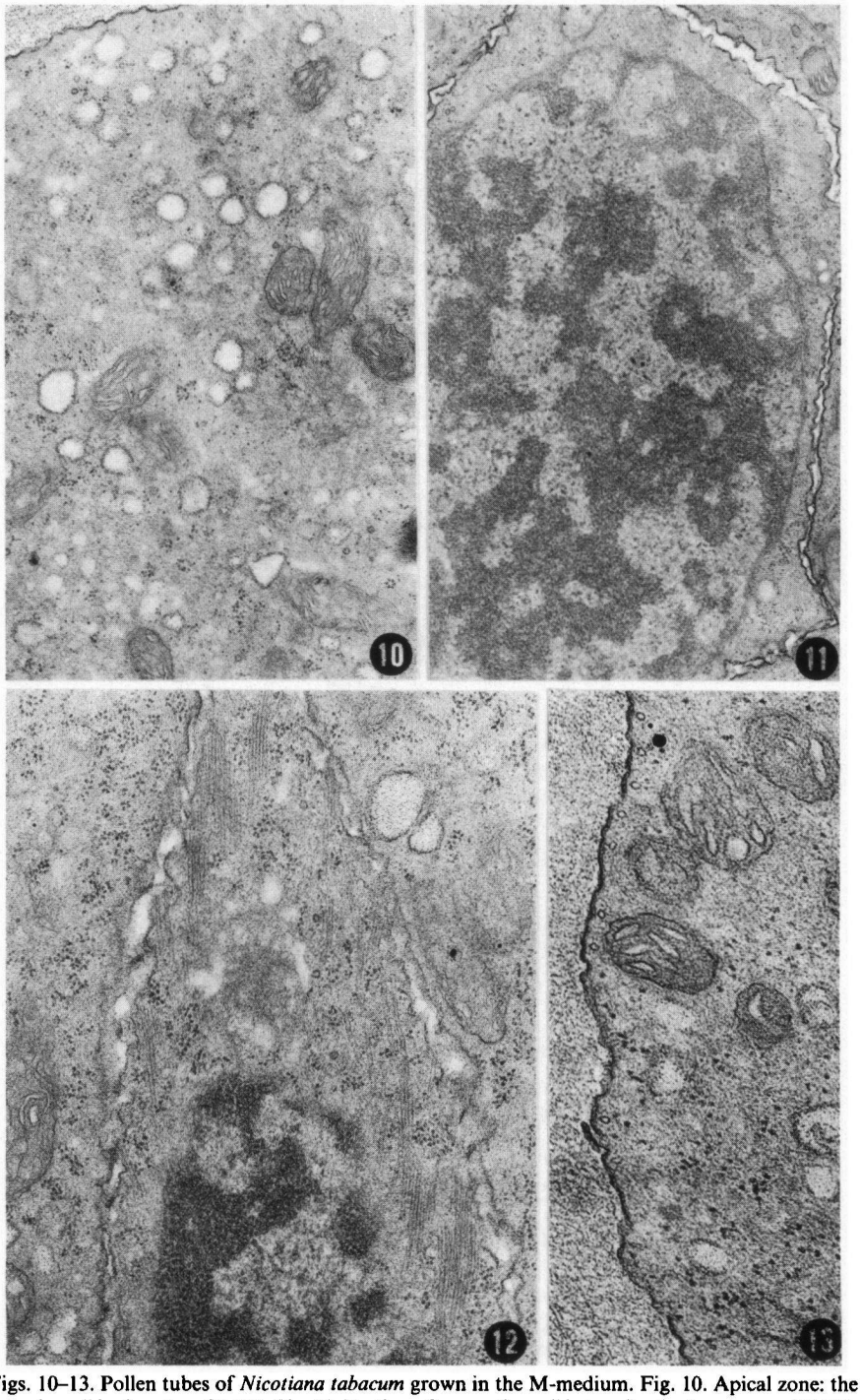
Figs. 1–3. Pollen tubes of *Nicotiana tabacum* grown in BK medium. Fig. 1. Zone of the tube in proximity of the tip: the callosic wall is absent. × 38.300. Fig. 2. Generative cell: the profile of the wall is not uniform. × 8.300. Fig. 3. Microtubular bundles in the generative cytoplasm. × 44.000.



Figs. 4–6. Pollen tubes of *Nicotiana tabacum* grown in PEG-medium. Fig. 4. Cytoplasm of the pollen tube near the apical zone. × 20.300. Fig. 5. Generative cell: the profile of generative wall appear regular; occasionally contact between the two membranes can be observed. × 13.800. Fig. 6. Network of microtubules inside the generative cytoplasm. × 38.000.



Figs. 7–9. Pollen tubes of *Nicotiana tabacum* grown in the W-medium. Fig. 7. Cytoplasm in the apical zone of the tube. × 32.000. Fig. 8. Longitudinal section of pollen tube containing a generative cell. Vesicles between the two membranes are present. × 8.000. Fig. 9. Bundles of microtubules in the generative cytoplasm. × 36.700.



Figs. 10–13. Pollen tubes of *Nicotiana tabacum* grown in the M-medium. Fig. 10. Apical zone: the callosic wall is absent. × 25.000. Fig. 11. Portion of generative cell in longitudinal section. × 20.000. Fig. 12. Bundles of microtubules in the generative cytoplasm. × 30.000. Fig. 13. Microtubules in the generative cytoplasm in proximity of the callosic wall. × 45.000.

In the generative cell just prior to germination, a close network of microtubules (*fig. 6*) which subsequently became organized in bundles, was observed. As in the case of the medium BK, no microtubles were visible in the vegetative cytoplasm.

3.3. W-medium

The pollen tubes grew rapidly in the first 3–4 hours. The tips were greatly enlarged. The pectocellulosic and callosic walls were clearly visible and the cellulosic fibrils seemed disposed in a disorderly manner at times. In the apical zone the plasma membrane was irregular; the cell organelles were well preserved (*fig. 7*). The generative cell wall had many vesicles which were occasionally found between the two membranes (*fig. 8*). The vegetative and generative cytoplasm had the same electrondensity. After germination, bundles of microtubules could be seen in the generative cell cytoplasm (*fig. 9*). No microtubles were noted in the vegetative cytoplasm.

3.4. M-medium

The pollen tubes grew rapidly in the first 3–4 hours. Tube tips were rarely enlarged, the organelles showed excellent preservation (*fig. 10*). As with the other culture media, the pectocellulosic and callosic pollen tube walls were clearly evident. The generative cell wall was uniform (*fig. 11*) and there were only occasional points of contact between the two membranes. The electrondensities of vegetative and generative cytoplasms were the same. Many microtubules organized in bundles were visible in the generative cell (*fig. 12*). In many pollen tubes cultivated in this medium, microtubules close to the callosic wall were clearly observed (*fig. 13*).

4. DISCUSSION

The present study indicates that the four culture media examined do not cause substantial differences in pollen and pollen tube behaviour except for the percentage of germination and the number of generative cells in division.

Ultrastructural examination reveals that the different media do not provoke significant morphological differences, although the generative cell wall may show a variable degree of vesiculation especially in PEG. This phenomenon is probably due to the different osmoregulation conditions of this medium (HONG-QI & CROES 1982). It is interesting to note that in all the media, microtubules as described in other species (CRESTI et al. 1984), are clearly visible in the generative cell cytoplasm. Although the existence of cytoskeletal apparatus in the pollen tube has already been described (FRANKE et al. 1972, DERKSEN et al. 1985) in the present study only the pollen tubes grown in M-medium showed microtubules in the vegetative cell.

The different percentage of generative cell divisions in the different media is an interesting finding. The highest values occur in M and W-media but are

still less than those obtained in *Tradescantia* (LAFLEUR et al. 1981).

The differences in percentage germination are minimal; probably W and M are the more selective of the media studied because of their greater chemical complexity.

Without going into the physiological roles of the different chemical components in pollen tube growth, generative cell division and subsequent sperm formation, we conclude that media W and M better suit the aims of this study.

Further studies utilizing the two media (MW) together in different proportions combined with water potential and respiration rate measurements, in order to increasing generative cell division, are in progress in our laboratory.

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